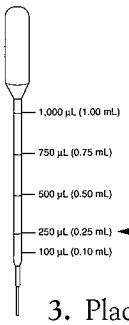
Transformations

Student Sheet

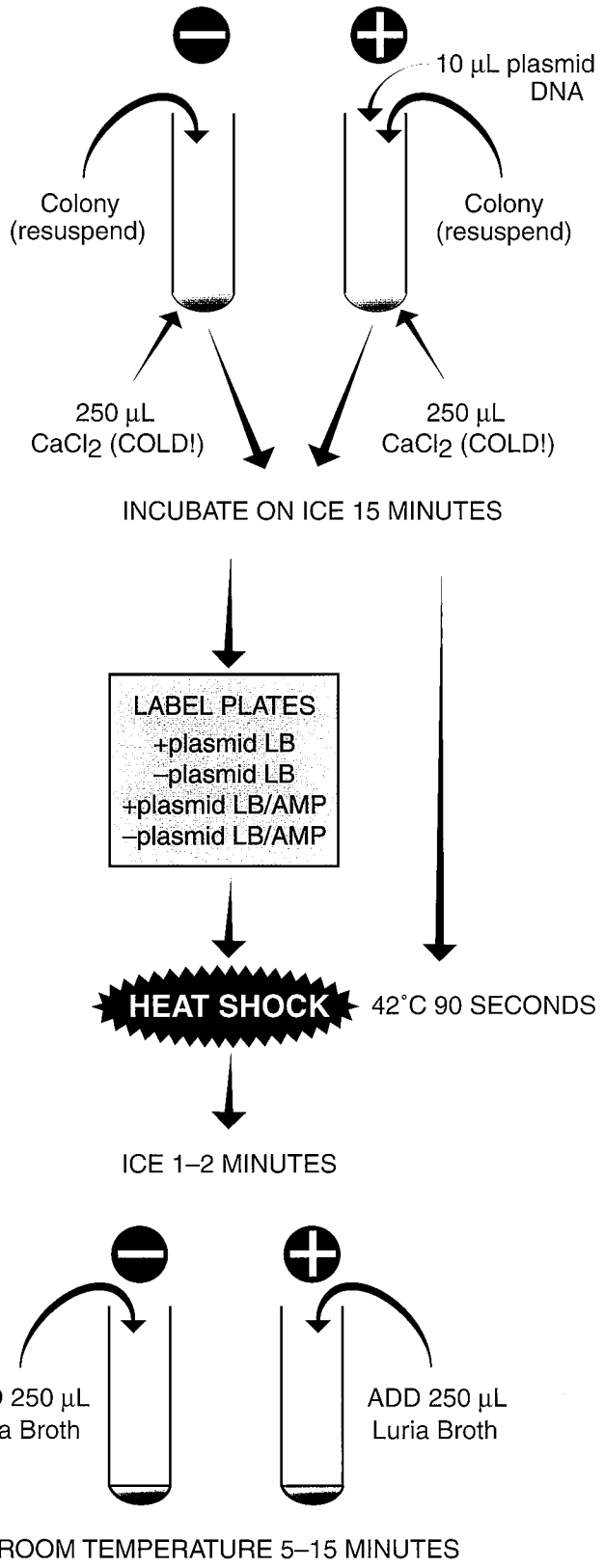
Laboratory Procedure for pGREEN

Name:

Date:



1. Mark one sterile 15-mL tube "+ plasmid." Mark another "–plasmid." (Plasmid DNA will be added to the "+plasmid" tube; none will be added to the "–plasmid" tube.)
2. Use a sterile transfer pipet to add 250 µL of ice-cold calcium chloride to each tube.
3. Place both tubes on ice.
4. Use a sterile plastic inoculating loop to transfer isolated colonies of *E. coli* from the starter plate to the +plasmid tube. The total area of the colonies picked should be equal in size to the top of a pencil eraser.
   a. Be careful not to transfer any agar from the plate along with the cell mass.
   b. Immerse the cells on the loop in the calcium chloride solution in the +plasmid tube and vigorously spin the loop in the solution to dislodge the cell mass. Hold the tube up to the light to observe that the cell mass has fallen off the loop.
5. **Immediately** suspend the cells by repeatedly pipetting in and out with a sterile transfer pipet. Examine the tube against light to confirm that no visible clumps of cells remain in the tube or are lost in the bulb of the transfer pipet. The suspension should appear milky white.
6. Return the +plasmid tube to ice. Transfer a mass of cells to the –plasmid tube and *suspend as described in steps 4 and 5 above.*
7. Return the –plasmid tube to ice. Both tubes should now be on ice.
8. Use a sterile plastic inoculating loop to add one loopful of plasmid DNA to the +plasmid tube. (When the DNA solution forms a bubble across the loop opening, its volume is 10 µL.) Immerse the loopful of plasmid DNA *directly into* the cell suspension and spin the loop to mix the DNA with the cells.
9. Return the +plasmid tube to ice and incubate both tubes on ice for 15 minutes.
10. While the tubes are incubating, label your media plates as follows and with your lab group name and date:
    a. Label one LB/Amp plate "+plasmid." This is an experimental plate.



b. Label the other LB/Amp plate "–plasmid." This is a negative control.

c. Label your LB plate either "+plasmid" or "–plasmid," according to your teacher's instructions. This is a positive control to test the viability of the cells after they have gone through the transformation procedure.

11. Following the 15-minute incubation on ice, "heat shock" the cells. Remove both tubes **directly from ice and immediately** immerse them in the 42°C water bath for 90 seconds. Gently agitate the tubes while they are in the water bath. Return both the tubes **directly** to ice for 1 or more minutes.

12. Use a sterile transfer pipet to add 250 μL Luria broth (LB) to each tube. Gently tap the tubes with your finger to mix the LB with the cell suspension. Place the tubes in a test-tube rack at room temperature for a 5- to 15-minute recovery.

13. Now you will remove some cells from each transformation tube and spread them on the plates. Cells from the –plasmid tube should be spread on the –plasmid plates, and cells from the +plasmid tube should be spread on the +plasmid plates.

14. Use a sterile transfer pipet to add 100 μL of cells from the –plasmid transformation tube to each appropriate plate. Using the procedure below, immediately spread the cells over the surface of the plate(s).

a. "Clam shell" (slightly open) the lids and carefully pour 4–6 glass beads onto each plate.

b. Use a back-and-forth shaking motion (not swirling round and round) to move the glass beads across the **entire** surface of the plate(s). This should evenly spread the cell suspension all over the agar surface.

c. When you finish spreading, let the plates rest for several minutes to allow the cell suspensions to become absorbed into the agar.

d. To remove the glass beads, hold each plate vertically over a container, clam shell the lower part of the plate, and tap out the glass beads into the container.

15. Use another sterile transfer pipet to add 100 μL of cell suspension from the +plasmid tube to each appropriate plate.

16. Immediately spread the cell suspension(s) as described in step 14.

17. Wrap the plates together with tape and place the plates upside down either in the incubator or at room temperature. Incubate them for approximately 24–36 hours in a 37°C incubator or 48–72 hours at room temperature.

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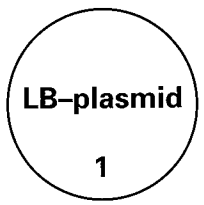
Data and Analysis for pGREEN

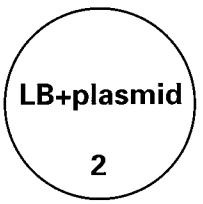
1. Predict your results. Write "yes" or "no," depending on whether you think the plate will show growth. Give the reason(s) for your predictions.
2. Observe the colonies through the petri plate lids. Do not open the plates.



Prediction:

Reason:

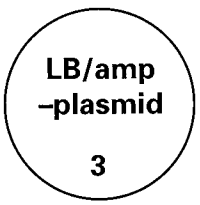
Observed Result:



Prediction:

Reason:

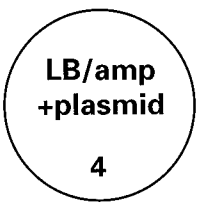
Observed Result:



Prediction:

Reason:

Observed Result:



Prediction:

Reason:

Observed Result:

3. Record your observed results in the spaces above. If your observed results differed from your predictions, explain what you think may have occurred.

4. Count the number of individual colonies and, using a permanent marker, mark each colony as it is counted. If the cell growth is too dense to count individual colonies, record "lawn."

LB+plasmid (Positive Control)

LB/Amp+plasmid (Experimental)

LB–plasmid (Positive Control)

LB/Amp–plasmid (Negative Control)

5. Compare and contrast the number of colonies on each of the following pairs of plates. What does each pair of results tell you about the experiment?

   a. LB+plasmid and LB–plasmid

   b. LB/Amp–plasmid and LB–plasmid

   c. LB/Amp+plasmid and LB/Amp–plasmid

   d. LB/Amp+plasmid and LB+plasmid

6. What are you selecting for in this experiment? (i.e., what allows you to identify which bacteria have taken up the plasmid?)

7. What does the phenotype of the transformed colonies tell you?

8. What one plate would you first inspect to conclude that the transformation occurred successfully? Why?

9. Transformation efficiency is expressed as the number of antibiotic-resistant colonies per μg of plasmid DNA. The object is to determine the mass of plasmid that was spread on the experimental plate and that was, therefore, responsible for the transformants (the number of colonies) observed.

   Because transformation is limited to only those cells that are competent, increasing the amount of plasmid used does not necessarily increase the probability that a cell will be transformed. A sample of competent cells is usually saturated with the addition of a small amount of plasmid, and excess DNA may actually interfere with the transformation process.

   a. Determine the total mass (in μg) of plasmid used. Remember, you used 10 μL of plasmid at a concentration of 0.005 μg/μL.

      total mass = volume × concentration

   b. Calculate the total volume of cell suspension prepared.

   c. Now calculate the fraction of the total cell suspension that was spread on the plate.

      volume suspension spread/total volume suspension = fraction spread

   d. Determine the mass of plasmid in the cell suspension spread.

      total mass plasmid (a) × fraction spread (c) = mass plasmid DNA spread

   e. Determine the number of colonies per μg plasmid DNA. Express your answer in scientific notation.

      colonies observed/mass plasmid spread (d) = transformation efficiency

10. What factors might influence transformation efficiency? Explain the effect of each factor you mention.

